INTEGRIN-αVβ3 AND FERROPTOSIS: A POWERFUL, NEW STRATEGY FOR MEDULLOBLASTOMA TREATMENT

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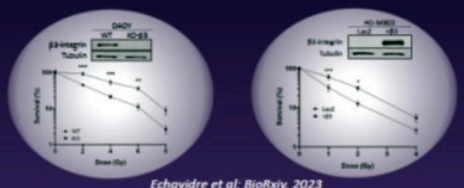
Medulloblastoma (MB) is the most frequent malignant pediatric brain tumor, localized in the posterior fossa of the brain. The standard care comprise resection surgery followed by radio- and chemotherapy. Relapse, characterized by a dismal prognosis, occurs in approximately 30% of cases and remains an area of unmet clinical need.

Our team showed that integrin-αvβ3, a member of the superfamily adhesion molecules, plays a key role in the radioresistance of MB. Consequently, the depletion of β3-subunit increased sensitivity of MB cells to radiotherapy. Interestingly, cell death observed thereby showed a very specific bubbling phenotype characteristic for ferroptotic cell death .



Echavidre et al; BioRxiv, 2023

Ferroptosis is a Reactive Oxygen Species (ROS)-dependent type of regulated cell death. The major event leading to ferroptosis is free iron-catalyzed oxidation of the lipids in the plasma membrane, resulting in the disruption of its integrity, cell bubbling, and finally death by explosion. Under physiological condition, it is prevented by the canonical Glutathione peroxidase 4 (GPX4)-glutathione (GSH) axis. In the laboratory settings ferroptosis can be prevented by lipophilic antioxidants, such as ferrostatin-1 (Fer-1) and Vitamin E (VitE).

Aim:

Investigated the effects of integrin-αvβ3 on the sensitivity of MB to ferroptosis.

1. INTEGRIN-αVβ3 PROTECTS MEDULLOBLASTOMA CELLS FROM RADIATION-INDUCED FERROPTOSIS

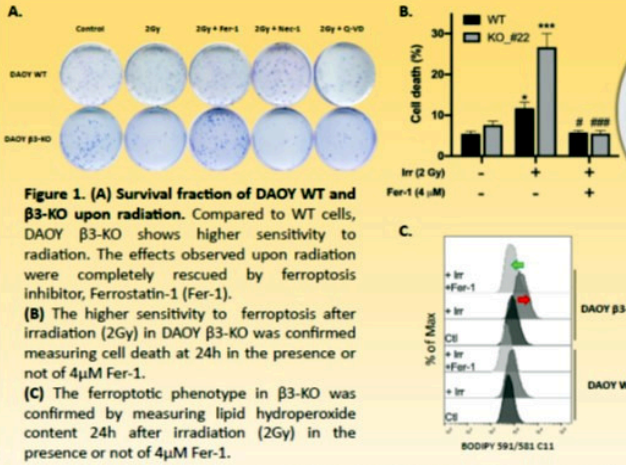


Figure 1. (A) Survival fraction of DAOY WT and β3-KO upon radiation. Compared to WT cells, DAOY β3-KO shows higher sensitivity to radiation. The effects observed upon radiation were completely rescued by ferroptosis inhibitor, Ferrostatin-1 (Fer-1).
(B) The higher sensitivity to ferroptosis after irradiation (2Gy) in DAOY β3-KO was confirmed measuring cell death at 24h in the presence or not of 4µM Fer-1.
(C) The ferroptotic phenotype in β3-KO was confirmed by measuring lipid hydroperoxide content 24h after irradiation (2Gy) in the presence or not of 4µM Fer-1.

*The results are presented as mean ± SEM, n = 3. * p < 0.05, *** p < 0.001, comparison with corresponding control. #p<0.05, ###p<0.001, comparison with corresponding cell line after irradiation.*

2. INTEGRIN-αVβ3 CONTROLS ANTI-FERROPTOTIC AXIS THROUGH ITS ACTION ON MTORC1

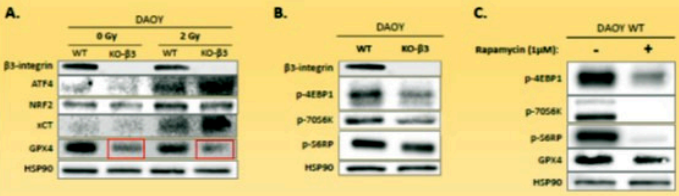
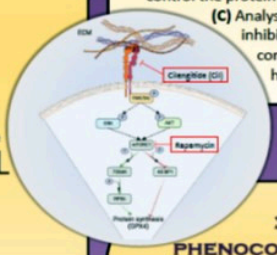


Figure 2. (A) Protein content of major players involved in ferroptosis prevention were measured, in DAOY WT/β3-KO, 24h post-radiation (2Gy). GPX4 protein level is significantly decrease in DAOY β3-KO comparing with WT (red squares), and this level was maintained low even after radiation. This might explain higher sensitivity of DAOY β3-KO to radiation in comparison with their WT counterparts.
(B) Downstream targets of integrin αvβ3 signalling were investigated. Analysis of mTORC1 activity was measure by investigating the content of phosphorylated form of S6 kinase (p-S6K), ribosomal protein S6 (p-RPS6) and eukaryotic translation initiation factor 4E binding protein 1 (p4E-BP1) in WT and β3-KO cells. These targets were decreased in DAOY β3-KO in comparison with their WT counterparts, suggesting that integrin-αvβ3 signaling might control the protein synthesis of GPX4 via regulating the activity of the mTORC1.
(C) Analysis of mTORC1 capacity to control the GPX4 protein synthesis after its inhibition with 1µM Rapamycin. Data unequivocally suggest that GPX4 content depends on the activity of the mTORC1, arguing in favour of our hypothesis.



3. PHARMACOLOGICAL INHIBITION PHENOCOPIED GENETIC DELETION OF INTEGRIN-αVβ3 - CLINICAL POTENTIAL OF CILENGITIDE -

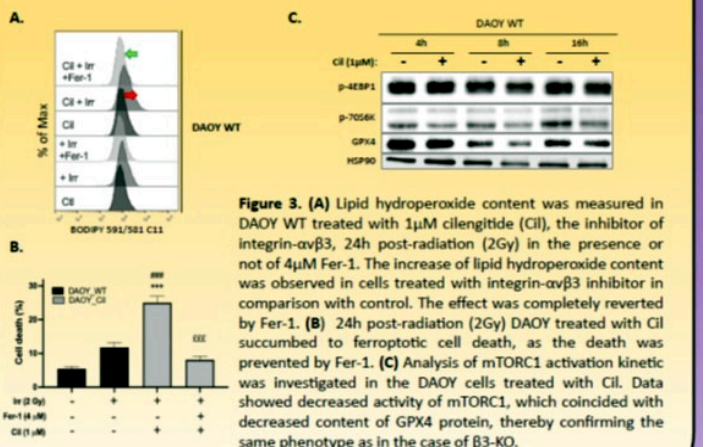
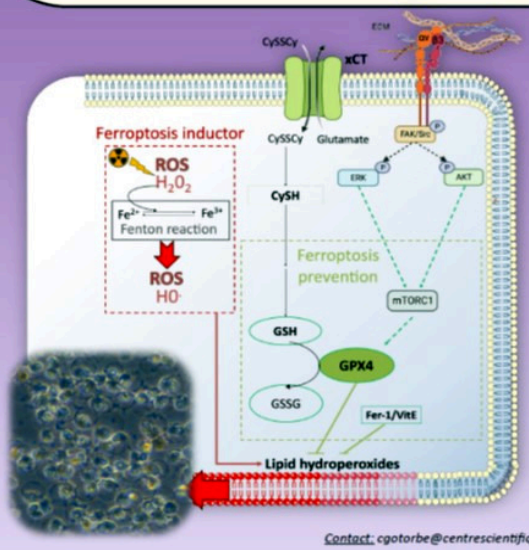


Figure 3. (A) Lipid hydroperoxide content was measured in DAOY WT treated with 1µM cilengitide (Cil), the inhibitor of integrin-αvβ3, 24h post-radiation (2Gy) in the presence or not of 4µM Fer-1. The increase of lipid hydroperoxide content was observed in cells treated with integrin-αvβ3 inhibitor in comparison with control. The effect was completely reverted by Fer-1. **(B)** 24h post-radiation (2Gy) DAOY treated with Cil succumbed to ferroptotic cell death, as the death was prevented by Fer-1. **(C)** Analysis of mTORC1 activation kinetic was investigated in the DAOY cells treated with Cil. Data showed decreased activity of mTORC1, which coincided with decreased content of GPX4 protein, thereby confirming the same phenotype as in the case of β3-KO.

*The results are presented as mean ± SEM, n = 3. ***p<0.001, comparison with irradiation condition in control conditions. ###p<0.001, comparison with irradiation without Cilengitide. £££p<0.001 , comparison with irradiation with Cilengitide.*



CONCLUSION

The signalling stemming from integrin-αvβ3 allows MB cells to maintain high expression of the main anti-ferroptotic player, GPX4, explaining their low sensitivity to radiation. This effect is most likely due to the effect of integrin-αvβ3 on the general cellular hub of nutrient sensor and protein synthesis, mechanistic target of rapamycin complex 1 (mTORC1).
These data clearly indicates that targeting integrin-αvβ3 can significantly impact MB cells radio-response, making it attractive target for anti-cancer therapy for MB patients.

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